

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
REQUEST FOR FILING NATIONAL PHASE OF
PCT APPLICATION UNDER 35 U.S.C. 371 AND 37 CFR 1.494 OR 1.495

To: Hon. Commissioner of Patents
 Washington, D.C. 20231



00909

TRANSMITTAL LETTER TO THE UNITED STATES
 DESIGNATED/ELECTED OFFICE (DO/EO/US)

Atty Dkt: P 280101 /BO-42135-JGD
M# /Client Ref.

From: Pillsbury Winthrop LLP, IP Group:

Date: April 5, 2001

This is a **REQUEST** for **FILING** a PCT/USA National Phase Application based on:

1. International Application <u>PCT/NL98/00579</u> ↑ country code	2. International Filing Date <u>8</u> <u>October</u> <u>1998</u> Day MONTH Year	3. Earliest Priority Date Claimed <u>8</u> <u>October</u> <u>1998</u> Day MONTH Year (use item 2 if no earlier priority)
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4. Measured from the earliest priority date in item 3, this PCT/USA National Phase Application Request is being filed within:

(a) ☐ 20 months from above item 3 date (b) ☒ 30 months from above item 3 date,

(c) Therefore, the due date (unextendable) is April 8, 2001

5. Title of Invention PEPTIDE-BASED CARRIER DEVICES FOR STELLATE CELLS

6. Inventor(s) POELSTRA, Klaas et al

Applicant herewith submits the following under 35 U.S.C. 371 to effect filing:

7. ☒ Please immediately start national examination procedures (35 U.S.C. 371 (f)).

8. ☐ A copy of the International Application as filed (35 U.S.C. 371(c)(2)) is transmitted herewith (file if in English but, if in foreign language, file only if not transmitted to PTO by the International Bureau) including:

a. ☐ Request;

b. ☐ Abstract;

c. pgs. Spec. and Claims;

d. sheet(s) Drawing which are ☐ informal ☐ formal of size ☐ A4 ☐ 11"

9. ☒ A copy of the International Application has been transmitted by the International Bureau.

10. A translation of the International Application into English (35 U.S.C. 371(c)(2))

a. ☒ is transmitted herewith including: (1) ☒ Request; (2) ☒ Abstract;

(3) 25 pgs. Spec. and Claims;

(4) 6 sheet(s) Drawing which are:

☐ informal ☒ formal of size ☒ A4 ☐ 11"

b. ☐ is not required, as the application was filed in English.

c. ☐ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.

d. ☐ Translation verification attached (not required now).

RE: USA National Phase Filing of PCT /NL98/00579

11. ☒ Please see the attached Preliminary Amendment
12. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., **before 18th month from first priority date above in item 3, are transmitted herewith (file only if in English) including:**
13. ☒ PCT Article 19 claim amendments (if any) have been transmitted by the International Bureau
14. ☐ Translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., of **claim amendments made before 18th month, is attached (required by 20th month from the date in item 3 if box 4(a) above is X'd, or 30th month if box 4(b) is X'd, or else amendments will be considered canceled).**
15. **A declaration of the inventor (35 U.S.C. 371(c)(4))**
 a. ☐ is submitted herewith ☐ Original ☐ Facsimile/Copy
 b. ☒ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.
16. **An International Search Report (ISR):**
 a. Was prepared by ☒ European Patent Office ☐ Japanese Patent Office ☐ Other
 b. ☒ has been transmitted by the international Bureau to PTO.
 c. ☒ copy herewith (2 pg(s).) ☒ plus Annex of family members (3 pg(s).)
17. **International Preliminary Examination Report (IPER):**
 a. ☒ has been transmitted (if this letter is filed after 28 months from date in item 3) in English by the International Bureau with Annexes (if any) in original language.
 b. ☒ copy herewith in English.
 c.1 ☒ IPER Annex(es) in original language ("Annexes" are amendments made to claims/spec/drawings during Examination) including attached amended:
 c.2 ☒ Specification/claim pages #1 - 5 claims # 1 - 26
 Dwg Sheets #
 d. ☒ Translation of Annex(es) to IPER **(required by 30th month due date, or else annexed amendments will be considered canceled).**
18. **Information Disclosure Statement** including:
 a. ☒ Attached Form PTO-1449 listing documents
 b. ☒ Attached copies of documents listed on Form PTO-1449
 c. ☒ A concise explanation of relevance of ISR references is given in the ISR.
19. ☐ **Assignment** document and Cover Sheet for recording are attached. Please mail the recorded assignment document back to the person whose signature, name and address appear at the end of this letter.
20. ☒ Copy of Power to IA agent.
21. ☐ **Drawings** (complete only if 8d or 10a(4) not completed): ___ sheet(s) per set: ☐ 1 set informal;
☐ Formal of size ☐ A4 ☐ 11"
22. Small Entity Status ☒ is **Not** claimed ☐ is claimed (**pre-filing confirmation required**)
 22(a) ___ (No.) Small Entity Statement(s) enclosed (since 9/8/00 Small Entity Statements(s) not essential to make claim)
23. **Priority** is hereby claimed under 35 U.S.C. 119/365 based on the priority claim and the certified copy, both filed in the International Application during the international stage based on the filing in (country) The Netherlands of:
- | | <u>Application No.</u> | <u>Filing Date</u> | | <u>Application No.</u> | <u>Filing Date</u> |
|-----|------------------------|--------------------|-----|------------------------|--------------------|
| (1) | PCT/NL98/00579 | October 8, 1998 | (2) | _____ | _____ |
| (3) | _____ | _____ | (4) | _____ | _____ |
| (5) | _____ | _____ | (6) | _____ | _____ |
- a. ☒ See Form PCT/IB/304 sent to US/DO with copy of priority documents. If copy has not been received, please proceed promptly to obtain same from the IB.
- b. ☐ Copy of Form PCT/IB/304 attached.

RE: USA National Phase Filing of PCT/NL98/00579

JC08 Rec'd PCT/PTO 05 APR 2001

24. Attached: Form PCT/IB/306

25. Per Item 17.c2, **cancel original** pages #_____, claims #_____, Drawing Sheets #**26. Calculation of the U.S. National Fee (35 U.S.C. 371 (c)(1)) and other fees is as follows:**Based on amended claim(s) per above item(s) ☐ 12, ☐ 14, ☒ 17, ☐ 25 (hilit) e)

Total Effective Claims	minus 20 =	x \$18/\$9	= \$0	966/967
Independent Claims	minus 3 =	x \$80/\$40	= \$0	964/965
If any proper (ignore improper) Multiple Dependent claim is present,		add \$270/\$135	+0	968/969

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(4)): →→ **BASIC FEE REQUIRED, NOW** →→→→A. If country code letters in item 1 are **not** "US", "BR", "BB", "TT", "MX", "IL", "NZ", "IN" or "ZA"

See item 16 re:

1. Search Report was <u>not prepared</u> by EPO or JPO -----	add \$1000/\$500		960/961
2. Search Report was prepared by EPO or JPO -----	add \$860/\$430	+860	970/971

SKIP B, C, D AND E UNLESS country code letters in item 1 are "US", "BR", "BB", "TT", "MX", "IL", "NZ", "IN" or "ZA"

(X) → <input type="checkbox"/> B. If <u>USPTO</u> did not issue <u>both</u> International Search Report (ISR) <u>and</u> (if box 4(b) above is X'd) the International Examination Report (IPER), -----	add \$970/\$485	+0	960/961
(only) (one) → <input type="checkbox"/> C. If <u>USPTO</u> issued ISR but not IPER (or box 4(a) above is X'd), -----	add \$710/\$355	+0	958/959
(these) (4) → <input type="checkbox"/> D. If <u>USPTO</u> issued IPER but IPER Sec. V boxes <u>not all</u> 3 YES, -----	add \$690/\$345	+0	956/957
→ <input type="checkbox"/> E. If international preliminary examination fee was paid to <u>USPTO</u> <u>and</u> Rules 492(a)(4) and 496(b) <u>satisfied</u> (IPER Sec. V <u>all</u> 3 boxes YES for <u>all</u> claims), -----	add \$100/\$50	+0	962/963

27. **SUBTOTAL =** \$86028. If Assignment box 19 above is X'd, add Assignment Recording fee of ----\$40 +0 (581)29. Attached is a check to cover the ----- **TOTAL FEES** \$860

Our Deposit Account No. 03-3975

Our Order No. 51079 280101

C#

M#



00909

CHARGE STATEMENT: The Commissioner is hereby authorized to charge any fee specifically authorized hereafter, or any missing or insufficient fee(s) filed, or asserted to be filed, or which should have been filed herewith or concerning any paper filed hereafter, and which may be required under Rules 16-18 and 492 (missing or insufficient fee only) now or hereafter relative to this application and the resulting Official document under Rule 20, or credit any overpayment, to our Account/Order Nos. shown above for which purpose a duplicate copy of this sheet is attached.

This CHARGE STATEMENT does not authorize charge of the issue fee until/unless an issue fee transmittal form is filed

Pillsbury Winthrop LLP
Intellectual Property Group

By Atty: Kendrew H. ColtonReg. No. 30368Sig: [Signature]Fax: (202) 822-0944

Atty/Sec: KHC/mhn

Tel: (202) 861-3606**NOTE:** File in duplicate with 2 postcard receipts (PAT-103) & attachments.

JC08 Rec'd PCT/PTO 05 APR 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF

Inventor(s): POELSTRA, Klaas et al

Filed: Herewith

Title: PEPTIDE-BASED CARRIER DEVICES FOR STELLATE CELLS

April 5, 2001

PRELIMINARY AMENDMENT

Hon. Commissioner of Patents
Washington, D.C. 20231

Sir:

Please amend this application as follows:

IN THE SPECIFICATION:

At the top of the first page, just under the title, insert

☒ --This application is the National Phase of International Application
PCT/NL98/00579 filed October 8, 1998 which designated the U.S.

and that International Application

☒ was ☐ was not published under PCT Article 21(2) in English.--

Respectfully submitted,

PILLSBURY WINTHROP LLP
Intellectual Property Group

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APPLICATION UNDER UNITED STATES PATENT LAWS

Atty. Dkt. No. PW 280101/BO-42135-JGD
(M#)

Invention: PEPTIDE-BASED CARRIER DEVICES FOR STELLATE CELLS

Inventor (s): POELSTRA, Klaas
BELJAARS, Eleonora
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This is a:

- ☐ Provisional Application
- ☐ Regular Utility Application
- ☐ Continuing Application
 - ☒ The contents of the parent are incorporated by reference
- ☒ PCT National Phase Application
- ☐ Design Application
- ☐ Reissue Application
- ☐ Plant Application
- ☐ Substitute Specification
 - Sub. Spec Filed _____
 - in App. No. _____ / _____
- ☐ Marked up Specification re
 - Sub. Spec. filed _____
 - In App. No. _____ / _____

SPECIFICATION

PEPTIDE-BASED CARRIER DEVICES FOR STELLATE CELLS

5 Background of the invention

The hallmark of fibrosis is the excessive deposit of extracellular matrix components caused by an increased synthesis and decreased degradation of matrix proteins, predominantly collagen type I and III. This process of fibrosis can occur in all kinds of organs such as the kidney (glomerulosclerosis or interstitial fibrosis), the skin (scar formation), the lung and also in the liver, where the end-stage of liver fibrosis is referred to as cirrhosis. The process also shares many characteristics with the formation of atherosclerotic plaques in arteries. Liver fibrosis leads to a deterioration of liver function, and eventually in complete liver failure, which is lethal if untreated. The process can be elicited by viruses (Hepatitis A, B and C), alcohol consumption, genetic disorders, or by chronic exposure to hepatotoxic agents. The incidence of this disease is very variable depending on the country. In the period 1985-1989, the incidence of liver cirrhosis in The Netherlands was 3.90 per 100,000 habitants per year, whereas this incidence in, for instance, France and Germany was 11.9 respectively 12.4. To date, no effective pharmacotherapeutic intervention is available for this disease. In the past decades liver transplantation has become a serious option for many patients but the costs, the availability of donor livers and the traumatic event of the transplantation itself hamper the application of such an operation in general practice. Pharmacological intervention would be a better option.

Hepatic stellate cells (HSC), also called Ito cells or fat storing cells strongly proliferate during the progression of the disease and they subsequently transform into myofibroblasts (MF). These cells are the major producers of collagens, glycoproteins, and proteoglycans in a diseased liver. Moreover, HSC and MF produce an array of mediators which activate other hepatic and inflammatory cells thus enhancing the fibrotic process. Therefore, HSC are an important target for anti-fibrotic therapy. However, in vivo studies indicate that anti-fibrotic drugs are not efficiently taken up by HSC and as a consequence, most drugs which showed potent anti-fibrotic activity in vitro, failed to exert any effect in vivo. At high doses such drugs often induce many side effects caused by extrahepatic distribution of the drug. Cell specific delivery is an option to solve these

problems. This can be accomplished by coupling drugs to carrier molecules, which are selectively taken up by the target cells. Liposomes are well known drug carriers but modified proteins can also be applied. Cell specific delivery of therapeutic and diagnostic agents to hepatocytes, endothelial and Kupffer cells has already been achieved by
5 modification of the sugar moieties of proteins or polymers. Coupling of galactose to, for instance, human serum albumin (HSA) leads to a specific accumulation of this neoglycoprotein in hepatocytes whereas addition of mannose to albumin causes uptake into Kupffer or endothelial cells. Increasing the net negative charge (for instance by succinylation of amine groups) results in uptake of the protein into endothelial cells via
10 scavenger receptors. For a comprehensive review on carrier devices for cell specific delivery of drugs see D.K.F. Meijer and G. Molema, Sem. in Liver Dis. 15: 202-256, 1995. The benefits of such carrier devices for the development of novel pharmacotherapeutic interventions for various diseases has been well recognized. However, a specific carrier for drugs to HSC, the most important cell in the
15 pathogenesis of liver fibrosis, has not been found yet.

Summary of the invention

The invention describes novel drug carriers which specifically accumulate in hepatic stellate cells (HSC). These carriers can be used for the targeting of all kinds of
20 therapeutic agents, preferably anti-fibrotic drugs to HSC. The carriers may also be applied for the visualization of HSC for diagnostic purposes. The basis of the invention lies in the coupling of small cyclic proteins (oligopeptides), that contain specific receptor recognising peptides (RRPs) to soluble or particle type carriers (core carriers). The use of such a conjugate as a tool for targeting purposes has not been described. The
25 target-receptors for these neo proteins, neo oligopeptides or oligopeptide carrier constructs are specific for HSC or are upregulated upon this cell type during the course of the disease. In the present study human serum albumin (HSA) is applied as the core-carrier, but the invention is not restricted to a specific protein or polymer. Each molecule with attachment sites for peptides is applicable as a carrier to HSC. The
30 invention describes conjugates which bind to the platelet derived growth factor (PDGF)-receptor and conjugates which attach to the collagen type VI receptor. Both types of receptors are present in relatively high amounts upon HSC and are well characterized. The respective receptor-binding ligands are known.

Also other receptors may be targeted using this new approach. Transforming Growth Factor β (TGF β), interleukin 1β (IL 1β), and Tumor Necrosis Factor α (TNF α) are other important mediators during chronic inflammatory processes and the receptors for these cytokines are upregulated upon HSC as well as upon endothelial cells and Kupffer cells in the liver. The ligands for these receptors are well characterized and similar to the PDGF-receptor or collagen VI-receptor recognizing proteins, cyclic peptides recognizing the binding site for these receptors can be prepared and coupled to a core-protein such as albumin. A relative accumulation of these conjugates can be expected into the target cell expressing the particular receptors. Most of the cytokines contain a RGD sequence (arg-gly-asp). This is the (putative) cell attachment site and in combination with additional amino acids it will determine the specificity for the individual cytokines and growth hormone receptors. Coupling this RGD sequence and accompanying amino acids to a carrier molecule using the approach described here is feasible. The invention also includes the preparation of oligopeptides in which more than one receptor recognizing domain for the same receptor are combined and peptide constructs in which different receptor recognizing domains for different types of receptors are combined. The particular oligopeptide constructs containing a single or more than one receptor recognizing domain can be used as such, as intrinsic active substances but also for the preparation of drug conjugates (pro-drugs) and be employed

to prepare larger drug carriers by coupling of the oligopeptides to either proteins, soluble and particulate polymeric carriers and lipid carriers (liposomes, neolipoproteins, micelles) that subsequently can be used for covalent binding and/or inclusion or association of therapeutic agents for the purpose of cell-specific drug targeting.

- 5 The application of such carriers is not limited to the treatment or diagnosis of fibrotic processes but also to other chronic and acute inflammatory processes such as, for instance, rheumatoid arthritis, Crohn's disease, colitis ulcerosa, glomerulonephritis and sepsis.

10 Detailed description of the invention

A compound according to the invention comprises a carrier molecule, said carrier molecule being linked to a further molecule, said further molecule being at least one cyclic peptide, said cyclic peptide comprising in the cyclic peptide portion thereof at least one sequence containing at least one specific receptor recognising peptide (RRP) and with the proviso the compound is not a naturally occurring receptor agonist or antagonist. Suitably in such a compound according to the invention the RRP is of a receptor specific for Hepatic Stellate Cells (HSC) or a receptor that is upregulated on HSC during disease.

20 The RRP can by way of example be the agonist or antagonist of a receptor selected from the group of receptors consisting of PDGF receptor, collagen type VI receptor, cytokine receptor such as TGF β , TNF α and interleukin 1 β .

Suitably when the RRP is of a collagen type VI receptor, cytokine receptor such as TGF β , TNF α and interleukin 1 β the cyclic portion of the cyclic peptide comprises at least the amino acid sequence RGD or KPT (lys-pro-thr) in the cyclic portion thereof. By way of example the cyclic portion of the cyclic peptide comprises at least an amino acid sequence selected from X*YRGDYX* (Xaa-(Xaa)_n-arg-gly-asp-(Xaa)_n-Xaa) and X*YKPTYX* (Xaa-(Xaa)_n-lys-pro-thr-(Xaa)_n-Xaa) wherein X* represents the location of cyclisation and Y represents at least one amino acid or a sequence of amino acids up to a length such that the receptor binding capacity of the cyclic peptide is retained. In a preferred embodiment X* represents the location of attachment to the carrier molecule. An embodiment illustrating the above when the receptor agonist is of a collagen type VI receptor has a cyclic portion of the cyclic peptide comprising the amino acid sequence X*GRGDSPX* (Xaa-gly-arg-gly-asp-ser-

pro-Xaa). Suitably it will comprise the sequence -cysteine-glycine-arginine-glycine-aspartic acid-serine-proline-cysteine.

Suitably when the receptor agonist is of a interleukin 1 beta receptor the cyclic portion of the cyclic peptide can comprise the amino acid sequence X*DKPTLX* (Xaa-asp-lys-pro-thr-lys-Xaa).

Alternatively when the receptor agonist is of a PDGF receptor the cyclic portion of the cyclic peptide can comprise the amino acid sequence X*SRNLIDCX* (Xaa-ser-arg-asn-leu-ile-asp-cys-Xaa), wherein X* represents the location of cyclisation. In a preferred embodiment X* represents the location of attachment to the carrier molecule. Such a compound will bind to the PDGF receptor alpha and beta subtypes. Suitably it will comprise the sequence -cysteine-serine-arginine-asparagine-leucine-isoleucine-aspartic acid-cysteine.

In any of the embodiments according to the invention that are described X* can be a cysteine residue.

Only some crucial amino acids for the cyclic peptides are provided here. The oligopeptide may be elongated without causing a change in the cellular distribution pattern in vivo. Cyclisation of these peptides can be achieved for example by a disulfide bond between both cysteine groups. The free amine (α -amine) in one cysteine residue can be used to couple the oligopeptide to the carrier molecule. For example to the amine groups in a core-molecule like albumin, using succinimide-acetyl thioacetate (SATA). Coupling of more than one oligopeptide to albumin can be readily done. Attachment of the cyclic peptides to a carrier molecule via a biodegradable spacer, causing local release of the cyclic peptides, is also feasible. The examples provided here describe conjugates with multiple oligopeptides per HSA molecule, leaving enough free reactive groups within the core-protein (hydroxyl, amine or sulphate groups) to attach additional drugs or other chemicals. These conjugates selectively accumulate in HSC of normal and diseased livers.

The cyclic portion of the cyclic peptide can suitably comprise multiple RRP sequences. The cyclic portion of the cyclic peptide can comprise multiple RRP sequences directed at at least two different types of receptors. Obviously they can also be directed at the same type of receptor. Combinations of various receptor agonist sequences are naturally also possible. Thus a compound according to the invention in any of the embodiments defined may comprise multiple cyclic peptides directed at the same or

different subtypes of receptors or may comprise multiple but similar oligopeptides that contain more than one identical or different RRP sequence directed at the same receptor or different receptors on the particular cell type respectively. By way of example a compound according to the invention, wherein the carrier molecule is linked to more than one cyclic peptide can suitably comprise 5-15 cyclic peptides as defined in any of the embodiments above.

A person skilled in the art will realise numerous types of carrier molecules can be applied. The carrier molecule can suitably be selected from endogenous plasma proteins e.g. albumin, lactoferrin, alkaline phosphatase, superoxide dismutase, alpha2 macroglobulin and fibronectin. They are to be pharmaceutically acceptable and of a size such that they preferably are not lost due to the renal excretion thereof. Such compounds are suitably larger than 5000 Daltons. Suitable examples of the carrier molecule can be selected from the group of carrier molecules consisting of proteins, oligo or polypeptides, immunoglobulins or parts thereof, oligonucleotides, disaccharides, polysaccharides, biodegradable synthetic polymers, liposomes, lipid particles, biocompatible polymers in the form of microspheres or nanoparticles. Quite suitably in a compound according to this aspect of the invention the carrier molecule is the endogenous plasma protein albumin. The immunoglobulins can be mono or polyclonal. Parts of immunoglobulins can comprise Fab' fragments or single chain Ig. Humanised antibodies and bispecific antibodies are envisaged. In the case of human administration carriers that occur naturally in humans are preferred. For the sake of easy linkage of the carrier molecule to the cyclic peptide the carrier molecule preferably comprises free reactive groups such as hydroxyl, amine or sulphate. The carrier molecule can suitably be linked to the cyclic peptide via a biodegradable spacer. The carrier molecule can itself be a drug, the activity of which is not impaired by linking the cyclic peptide to it.

In an alternative embodiment of the invention the carrier molecule in the compound can comprise additional drugs or chemicals linked thereto.

The invention also covers a pharmaceutical composition comprising a compound according to any of the aforementioned embodiments as targeting ingredient and any pharmaceutically acceptable carrier. A pharmaceutical composition according to the invention comprises a compound in any of the embodiments mentioned above as pharmaceutically active ingredient in combination with any pharmaceutically acceptable additional carrier. In an alternative embodiment the pharmaceutical composition can

further comprise a drug biodegradably attached to the compound. It is also possible for the compound to further comprise a diagnostic marker attached thereto. A pharmaceutical composition according to the invention will be in a pharmaceutical dosage form. Such a dosage form can comprise sprayable, injectable or infusable solutions or solids or dosage forms for pulmonary or other administration routes. Also a pharmaceutical composition according to the invention can be in a topical form. In the case of parenteral administration a systemically acceptable form should be composed. This means it can enter the bloodstream without causing clotting or inadmissibly toxic reactions.

The invention is also directed at application of a compound according to the invention in any of the abovementioned embodiments as active targeting ingredient for manufacturing a pharmaceutical composition according to the invention for therapy, prophylaxis or diagnosis of chronic diseases. Examples from this group consist of fibrotic disease, sclerotic disease and chronic or acute inflammatory processes such as glomerulosclerosis, interstitial fibrosis, atherosclerosis, rheumatoid arthritis, Crohns disease, colitis ulcerosa, glomerulonephritis, lung fibrosis and sepsis. Suitably use of a compound according to the invention as active targeting ingredient for manufacturing a pharmaceutical composition according to the invention for therapy, prophylaxis or diagnosis of a disease related to proliferation of HSC is also envisaged as forming a particularly useful application to be covered by the invention. A method of targeting HSC, said method comprising administration in a pharmaceutically acceptable amount and form of a compound or a pharmaceutical composition according to the invention to a subject or a tissue sample of a subject is covered by the invention. The person skilled in the art will adjust the dosage to be applied to the manner of application, size, weight, state of health etc of the subject to which administration is to occur. Administration can occur in any manner known per se for administration of medicament.

The invention also covers a method of therapy, diagnosis or prophylaxis of a disease related to HSC, said method comprising administration in a pharmaceutically acceptable amount and form of a compound or a pharmaceutical composition according to the invention to a subject or a tissue sample of a subject. Particularly such disease can be one selected from the group consisting of fibrotic disease, sclerotic disease and chronic or acute inflammatory processes such as glomerulosclerosis, interstitial fibrosis, lung fibrosis, atherosclerosis, rheumatoid arthritis, Crohns disease, colitis ulcerosa,

glomerulonephritis and sepsis. The method comprises administration in a pharmaceutically acceptable amount and form of the compound or pharmaceutical composition according to the invention to a subject or a tissue sample of a subject. The person skilled in the art will adjust the dosage to be applied to the manner of application, size, weight, state of health etc of the subject to which administration is to occur. Administration can occur in any manner known per se for administration of therapeutic agents.

This further aspect of the invention will be illustrated but not limited in the following examples.

10

EXAMPLE 1

Normal rats and rats with liver fibrosis (3 weeks after bile duct ligation) received an intravenous injection of 10 mg/kg b.w. PDGF receptor-binding peptides conjugated with HSA. Based upon the results of the organ distribution studies with radiolabeled conjugates (figure 1), rats were sacrificed after ten minutes and samples of the liver and bone (from ribs, front paw, rear paw and the back) were removed for histochemical examination. No accumulation of this HSA-peptide conjugate was detectable in bone samples, whereas abundant staining was found in tissue samples. Upon cryostat sections of these livers double stainings were performed with anti-HSA antibodies and antibodies against Kupffer cells (ED1), endothelial cells (RECA-1), myofibroblasts (anti-actin antibodies) or hepatic stellate cells (desmin and GFAP antibodies). Subsequently, the number of double positive cells (HSA+ and cell marker+) were counted and related to the total number of HSA positive cells in the same area. Results of the quantitative evaluation of the carrier uptake in the liver are summarized in table 1.

25

Table 1: Relative accumulation of HSA modified with collagen VI-receptor recognising peptides (pCVI-HSA) or PDGF receptor-recognising peptides (pPB-HSA) in non-parenchymal cells of the liver. The number of HSA-positive cells was related to the number of cells double-positive for HSA and a HSC marker (desmine), or a EC marker (HIS 52), or a KC marker (ED2) or a PC marker (glycogen).

30

	% HSC	% EC	% KC	PC
pCVI-HSA	73 ± 14	30 ± 10	16 ± 11	-
pPB-HSA	72 ± 18	16 ± 6	11 ± 6	+

5 HSC = hepatic stellate cells, EC = endothelial cells, KC = Kupffer cells, PC = parenchymal cells

EXAMPLE 2

10 Normal rats and rats with liver fibrosis (3 weeks after bile duct ligation) received an intravenous injection of 10 mg/kg b.w. collagen VI receptor-binding peptides conjugated with HSA. Based upon the results of the organ distribution studies with radiolabeled conjugates (figure 2), rats were sacrificed after ten minutes and samples of the liver and bone (from ribs, front paw, rear paw and the back) were removed for
15 histochemical examination. No accumulation of this HSA-peptide conjugate was detectable in bone samples, whereas abundant staining was found in tissue samples. Upon cryostat sections of these livers double stainings were performed with anti-HSA antibodies and antibodies against Kupffer cells (ED1), endothelial cells (RECA-1), myofibroblasts (anti-actin antibodies) or hepatic stellate cells (desmin and GFAP
20 antibodies). Subsequently, the number of double positive cells (HSA+ and cell marker+) were counted and related to the total number of HSA positive cells in the same area. Results of the quantitative evaluation of the carrier uptake in the liver are summarized in table 1.

25

EXAMPLE 3

Another cyclic oligopeptide recognizing the PDGF-receptor can be described as follows:

30

-cysteine-arginine-lysine-lysine-proline-cysteine- (C*RKKPC*),

where the cysteines (C*) represent the cyclisizing residues.

Fig. 1: Organ distribution of human serum albumin (HSA) conjugated with 10 to 12 cyclic oligopeptides recognizing the PDGF-receptor in normal rats [figure A] and in rats with liver fibrosis induced by bile duct ligation (3 weeks after the operation) [fig.B]. Figure C represents the organ distribution of unmodified HSA. Organs were removed 10 minutes after intravenous administration of radiolabeled (^{125}I) protein and analyzed using a gamma-counter. The results are expressed as the mean \pm SD (n=3 per group). Note the accumulation of modified HSA in livers of normal and diseased rats.

Fig. 4: In vitro displacement of radiolabeled PDGF-BB from its receptor upon 3T3-fibroblasts by HSA-PDGF receptor-binding peptide conjugates (pPB-HSA, closed blocks), HSA (open blocks) or uncoupled PDGF-receptor binding peptides (pPB, open circles). Note the strong inhibition of binding of native PDGF to fibroblasts induced by the modified HSA, but not by native HSA or the oligopeptides alone.

The invention in a further aspect is directed at a compound capable of recognising and binding a mannose 6 phosphate receptor said compound comprising a carrier molecule linked to a molecule capable of recognising and capable of binding mannose-6-phosphate receptor, said molecules recognising and capable of binding mannose-6-phosphate receptor being present on the carrier molecule in at least an amount sufficient to occupy at least 20% of the carrier molecule linking sites for said molecules recognising and capable of binding mannose-6-phosphate receptor, with the proviso the compound is not latent tumor growth factor beta, thyroglobulin or a lysosomal protein. The latter are known proteins that are also known to comprise terminal mannose 6 phosphate groups and as such will bind to the mannose 6 phosphate receptor. They are excluded as compounds according to the invention. The substitution degree can be higher than 30% even as high as 40 or 50%. A suitable example of the molecule capable of recognising and capable of binding mannose-6-phosphate receptor is

mannose 6 phosphate.

In a compound according to this aspect of the invention the carrier molecule can be selected from the group consisting of proteins, oligo or polypeptides, immunoglobulins or parts thereof, oligonucleotides, disaccharides, polysaccharides, biodegradable synthetic polymers, liposomes, lipid particles, biocompatible polymers in the form of microspheres or nanoparticles. The carrier molecule can suitably be selected from endogenous plasma proteins e.g. albumin, lactoferrin, alkaline phosphatase, superoxide dismutase, alpha2 macroglobulin and fibronectin. The immunoglobulins can be mono or polyclonal. Parts of immunoglobulins can comprise Fab' fragments or single chain Ig. Humanised antibodies and bispecific antibodies are envisaged. Quite suitably in a compound according to this aspect of the invention the carrier molecule is the endogenous plasma protein albumin. A person skilled in the art will realise numerous types of carrier molecules can be applied. They are to be pharmaceutically acceptable and of a size such that they preferably are not lost due to the renal excretion thereof. Such compounds are suitably larger than 50000 Daltons.

Quite specifically in a preferred embodiment of a compound according to this aspect of the invention at least 10 molecules capable of recognising and capable of binding mannose-6-phosphate receptor are linked to the carrier molecule. The carrier based upon macromolecules substituted with mannose-6-phosphate residues with substitution of more than 10 mannose-6-phosphate residues per macromolecule has been found exceptionally appropriate for proper targeting. The carrier molecule was human serum albumin.

The invention also covers a pharmaceutical composition comprising a compound according to any of the aforementioned embodiments of the further aspect of the invention disclosed as targeting ingredient and any pharmaceutically acceptable carrier. A pharmaceutical composition according to the invention comprises a compound in any of the embodiments mentioned above as pharmaceutically active ingredient in combination with any pharmaceutically acceptable additional carrier. In an alternative embodiment the pharmaceutical composition can further comprise a drug biodegradably attached to the compound. It is also possible for the compound to further comprise a diagnostic marker attached thereto. A pharmaceutical composition according the invention will be in a medicinal dosage form. Such a dosage form can comprise sprayable, injectable or infusable solutions or solids or dosage forms for pulmonary or

The invention is also directed at application of a compound according to the invention in any of the abovementioned embodiments as active targeting ingredient for manufacturing a pharmaceutical composition according to the further aspect of the invention just mentioned for therapy, prophylaxis or diagnosis of a disease selected from the group of chronic diseases, for example fibrotic disease, sclerotic disease and chronic or acute inflammatory processes such as glomerulosclerosis, interstitial fibrosis, lung fibrosis, atherosclerosis, rheumatoid arthritis, Crohns disease, colitis ulcerosa, glomerulonephritis and sepsis.

The invention is also directed at application of a compound according to the invention in any of the abovementioned embodiments as active targeting ingredient for manufacturing a pharmaceutical composition according to the further aspect of the invention just mentioned for therapy, prophylaxis or diagnosis of any of the following pathological conditions; cell proliferation associated pathology e.g. tumors, a disease related to proliferation of HSC, fibroblast proliferation associated pathology, endothelial cell proliferation associated pathology and osteoblast proliferation associated pathology. The invention also covers a method of targeting proliferating cells, preferably tumor cells, HSC, fibroblasts, endothelial cells and osteoblasts said method comprising administration in a pharmaceutically acceptable amount and form of a compound or a pharmaceutical composition according to the aspect of the invention just described to a subject or a tissue sample of a subject. In an alternative embodiment it also covers a method of targeting proliferating cells, preferably tumor cells, HSC, fibroblasts, endothelial cells and osteoblasts said method comprising administration in a pharmaceutically acceptable amount and form of a compound or a pharmaceutical composition according to the further aspect of the invention just described to a subject or a tissue sample of a subject. Alternatively it covers a method of therapy, diagnosis or prophylaxis of a disease related to proliferating cells, preferably tumor cells, HSC, fibroblasts, endothelial cells and osteoblasts, said method comprising administration in a pharmaceutically acceptable amount and form of a compound or a pharmaceutical composition according to the further aspect of the invention to a subject or a tissue sample of a subject. Specifically it

covers a method of therapy, diagnosis or prophylaxis of a disease related to HSC, said method comprising administration in a pharmaceutically acceptable amount and form of a compound or a pharmaceutical composition as described for the further aspect of the invention to a subject or a tissue sample of a subject. A method of therapy, prophylaxis
5 or diagnosis of a disease selected from the group consisting of fibrotic disease, sclerotic disease and chronic or acute inflammatory processes such as glomerulosclerosis, interstitial fibrosis, lung fibrosis, atherosclerosis, rheumatoid arthritis, Crohns disease, colitis ulcerosa, glomerulonephritis and sepsis, said method comprising administration in a pharmaceutically acceptable amount and form of a compound or a pharmaceutical
10 composition as described for the further aspect of the invention to a subject or a tissue sample of a subject also falls within the scope of the invention.

This further aspect of the invention will be illustrated but not limited in the following examples.

15 EXAMPLE 5

Mannose 6-phosphate was covalently coupled to human serum albumin (HSA) in two steps. First, p-nitrophenyl- α -D-mannopyranoside (Sigma, St. Louis, USA) was phosphorylated according to standard procedures. The molecular weight (MW: 381) and purity of the obtained crystalline product p-nitrophenyl-6-phospho- α -D-mannopyranoside was verified by mass spectrometry. Subsequently, the nitro-group was
20 reduced with 10% palladium on active carbon (Aldrich Chemie GmbH, Steinheim, Germany) under hydrogen atmosphere of 1 atm. The obtained product p-aminophenyl-6-phospho- α -D-mannopyranoside was coupled to HSA by activation with thiophosgene. By variations in the molar ratio HSA:
25 p-nitrophenyl-6-phospho- α -D-mannopyranoside, a series of neoglycoproteins (M6P_x-HSA) were obtained, x = 2, 4, 10, 21, or 28. The M6P_x-HSA preparations were further purified and characterized according to standard procedures.

A tracer dose of modified HSA (¹²⁵I labelled) was intravenously administered to normal and fibrotic rats (three weeks after bile duct ligation). Ten minutes after injection of
30 these compounds, rats were sacrificed and all organs were removed. As can also be seen in figure 5, the degree of substitution of mannose 6-phosphate to HSA strongly influenced liver uptake. HSA with a low degree of sugar loading (x=2-10) accumulated for 2 \pm 1% to 9 \pm 0.5% in fibrotic rat livers, while the rest of the dose remained in the

circulation. An increase in the molar ratio of M6P:HSA up to 28 caused a gradual increase in liver accumulation (to $59 \pm 9\%$ of the dose).

In addition, the intrahepatic distribution of modified HSA was examined immunohistochemically. Modified HSA was administered to rats (10 mg/kg b.w.) and 10 minutes after the injection samples from the liver, spleen, kidney, and bone were histochemically examined. We observed that the more mannose 6-phosphate was linked to HSA, the higher the uptake was in HSC. Quantitative evaluation of liver sections ten minutes after administration of modified HSA revealed that M6P10-HSA accumulated for $19 \pm 10\%$ in HSC. In contrast, $69 \pm 12\%$ of the intrahepatic staining for M6P28-HSA was found in HSC, whereas $20 \pm 6\%$ was found in Kupffer cells and $17 \pm 6\%$ in endothelial cells. No uptake was detected in hepatocytes and bile duct epithelial cells. Also no staining for modified HSA was found in other organs.

EXAMPLE 6

M6P₂₁-bovine serum albumin (BSA) and M6P₂₈-HSA, synthesized and characterized according to standard procedures, were radiolabeled with ¹²⁵I. The intrahepatic uptake of these neo-glycoproteins was measured in human liver slices. These slices (± 10 mg liver tissue with a thickness of approximately 10 cells) were obtained from patients with normal liver function and from cirrhotic patients. Significant intrahepatic accumulation of radiolabeled BSA and HSA derivatives was found within one hour after co-incubation with these slices, whereas unmodified BSA or HSA was not taken up by the human tissue samples (see figure 6).

EXAMPLE 7

Pyrrolidine-dithiocarbamate (PDTC, which is an inhibitor of the transcription factor NF-kappaB) was attached to M6P₂₈-HSA by coupling the carboxylic groups of PDTC to lysine groups of HSA according to standard procedures. This compound was administered to rats with liverfibrosis induced by bile duct ligation. Rats receiving this conjugate 1, 3 and 5 days after the bile duct ligation displayed less proliferation of HSC in the parenchymal area at day 7 as compared to rats receiving no treatment or PDTC alone after induction of fibrosis. HSC were demonstrated in cryostat sections with anti-desmine and anti-Glial Fibrillar Acidic Protein (GFAP) antibodies and standard indirect immunoperoxidase techniques.

Note the high accumulation of neo-glycoprotein in human liver slices as compared to native HSA.

1. Method for targeting cells involved in sclerotic and/or fibrotic diseases in a tissue sample of a subject using a carrier molecule, said carrier molecule being linked to at least one further molecule, said further molecule being selected from the group comprising:
 - a cyclic peptide comprising the amino acid sequence RGD
 - a cyclic peptide comprising the amino acid sequence KPT
 - a cyclic peptide comprising the amino acid sequence RKKP
 - a cyclic peptide comprising the amino acid sequence SRNLIDC
- 10 - a molecule capable of recognising and binding mannose-6-phosphate receptor and at least an amount that is equivalent to at least 10 molecules capable of recognising and capable of binding mannose-6-phosphate receptor linked to HSA are linked to the carrier molecule.
- 15 2. Method for targeting cells involved in sclerotic and/or fibrotic diseases in a subject using, in a pharmaceutically acceptable amount and form a carrier molecule, said carrier molecule being linked to at least one further molecule, said further molecule being selected from the group comprising:
 - a cyclic peptide comprising the amino acid sequence RGD
 - 20 - a cyclic peptide comprising the amino acid sequence KPT
 - a cyclic peptide comprising the amino acid sequence RKKP
 - a cyclic peptide comprising the amino acid sequence SRNLIDC
 - a molecule capable of recognising and binding mannose-6-phosphate receptor and at least an amount that is equivalent to at least 10 molecules capable of recognising and
 - 25 capable of binding mannose-6-phosphate receptor linked to HSA are linked to the carrier molecule.
3. Method according to claim 1 or 2 wherein the cells comprise at least one target receptor specific for Hepatic Stellate Cells (HSC) or a receptor that is upregulated on HSC
- 30 during disease.

- AMENDED SHEET

11. Compound according to claim 10 wherein said further molecule is X*GRGDSPX*, wherein X* represents the location of cyclisation.

5 12. Compound for use in a method according to any of claims 1-7 said compound being a carrier molecule linked to at least one further molecule said further molecule being X*YKPTYX*, wherein X* represents the location of cyclisation and Y represents at least one amino acid or a sequence of amino acids up to a length such that the target receptor binding capacity of the further molecule is retained.

10

13. Compound according to claim 12 wherein said further molecule is X*DKPTLX*, wherein X* represents the location of cyclisation.

14. Compound for use in a method according to any of claims 1-7 said compound being a carrier molecule linked to at least one further molecule said further molecule being X*SRNLDCX*, wherein X* represents the location of cyclisation.

15. Compound for use in a method according to any of claims 1-7 said compound being a carrier molecule linked to at least one further molecule said further molecule being X*RKKPX*, wherein X* represents the location of cyclisation.

20

16. Compound according to any of claims 10-15 wherein X* is a cystein residue.

17. Compound according to any of claims 10-16 wherein X* represents the location of cyclisation and attachment to the carrier molecule.

25

18. Compound according to any of the claims 8-17 wherein of the further molecule the cyclic portion of the cyclic peptide comprises multiple receptor binding sequences.

19. Compound according to any of the claims 8-18 wherein of the further molecule the cyclic portion of the cyclic peptide comprises multiple receptor binding sequences directed at at least two different types of receptors.

30

20. Compound according to any of the claims 8-19, wherein the further molecule comprises multiple cyclic peptides directed at the same or different types of receptors.
21. Compound according to any of the claims 8-20, wherein the carrier molecule is
 5 selected from the group of carrier molecules consisting of proteins, oligo or polypeptides, immunoglobulins or parts thereof, oligonucleotides, disaccharides, polysaccharides, biodegradable synthetic polymers, liposomes, lipid particles, biocompatible polymers in the form of microspheres or nanoparticles, endogenous plasma proteins e.g. albumin, lactoferrin, alkaline phosphatase, superoxide dismutase, alpha2 macroglobulin and
 10 fibronectin.
22. Compound according to any of the claims 8-21, wherein the carrier molecule comprises additional drugs or chemicals linked thereto.
- 15 23. Compound according to any of the claims 8-23 wherein the carrier molecule comprises a diagnostic marker attached thereto.
24. Pharmaceutical composition comprising a compound according to any of claims 8-23 as targeting ingredient and one or more pharmaceutically acceptable carriers.
 20
25. Use of a compound according to claim 8-23 for in vitro diagnosis of a sclerotic and/or fibrotic disease in particular for in vitro diagnosis of a disease selected from the group consisting of liver fibrosis, in particular cirrhosis, kidney fibrosis, in particular
 25 glomerulosclerosis and interstitial fibrosis, lung fibrosis, atherosclerosis and chronic or acute inflammatory processes such as rheumatoid arthritis, Crohns disease, colitis ulcerosa, glomerulonephritis, sepsis and tumor-cell proliferation associated pathology, fibroblast proliferation associated pathology, endothelial cell proliferation associated pathology and osteoblast proliferation associated pathology with the proviso the compound *cyclo*[-D-Val-Arg-Gly-Asp-Glu(-εAhx-Tyr-Cys-NH)-] linked to BSA is not
 30 used in a cell adhesion assay for endothelial cells.
26. Use of a compound according to claim 8-23 or a pharmaceutical composition according to claim 24 for in vivo diagnosis, prophylaxis and/or therapy of a sclerotic

- and/or fibrotic disease in particular for in vitro diagnosis of a disease selected from the group consisting of liver fibrosis, in particular cirrhosis, kidney fibrosis, in particular glomerulosclerosis and interstitial fibrosis, lung fibrosis, atherosclerosis and chronic or acute inflammatory processes such as rheumatoid arthritis, Crohns disease, colitis
- 5 ulcerosa, glomerulonephritis, sepsis and tumor-cell proliferation associated pathology, fibroblast proliferation associated pathology, endothelial cell proliferation associated pathology and osteoblast proliferation associated pathology.

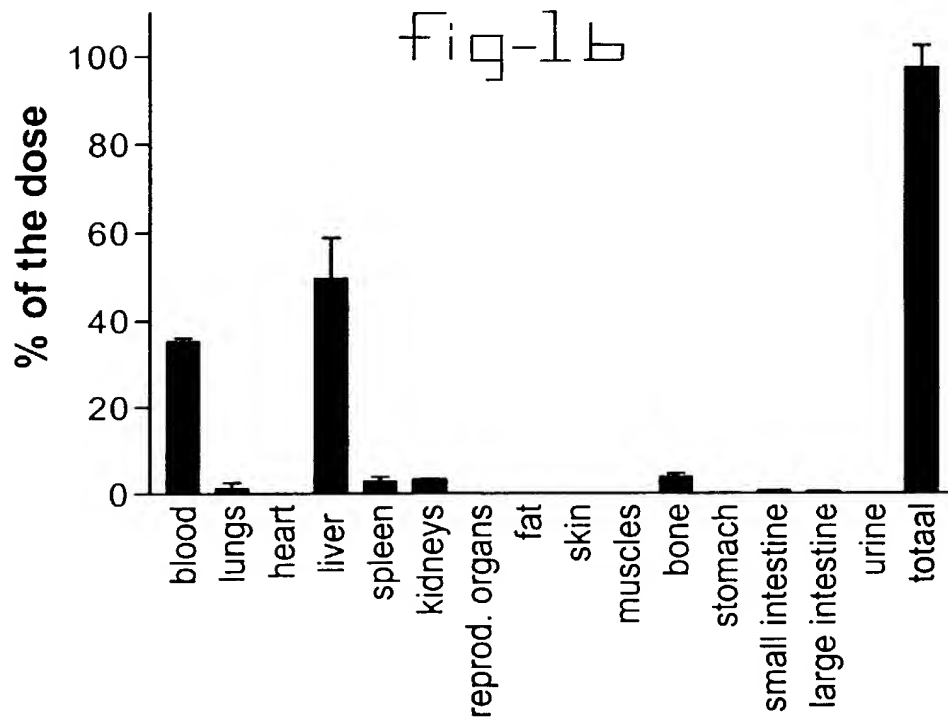
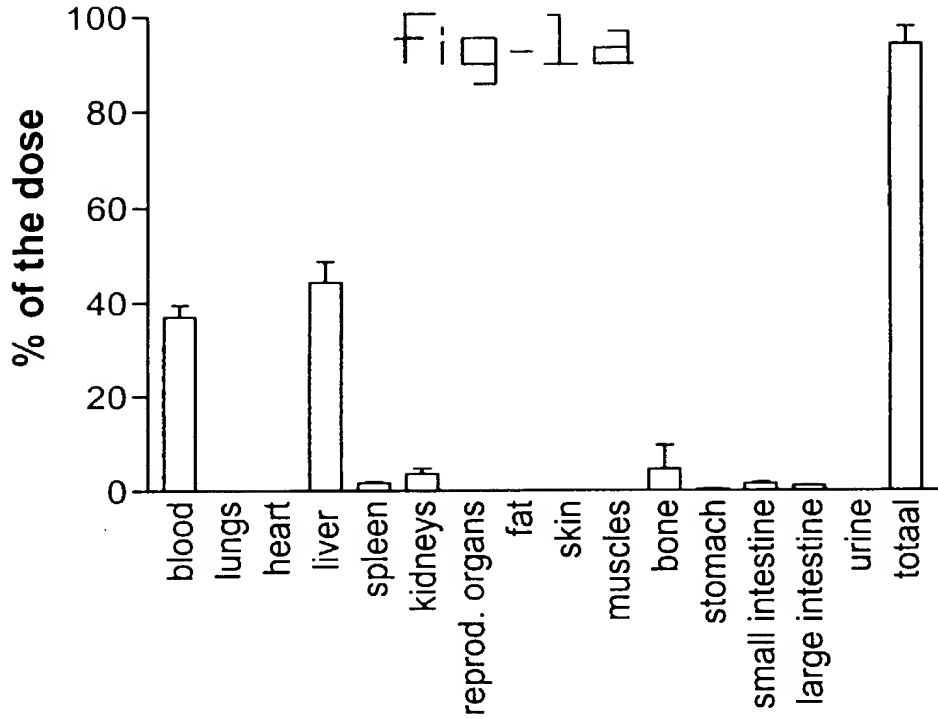
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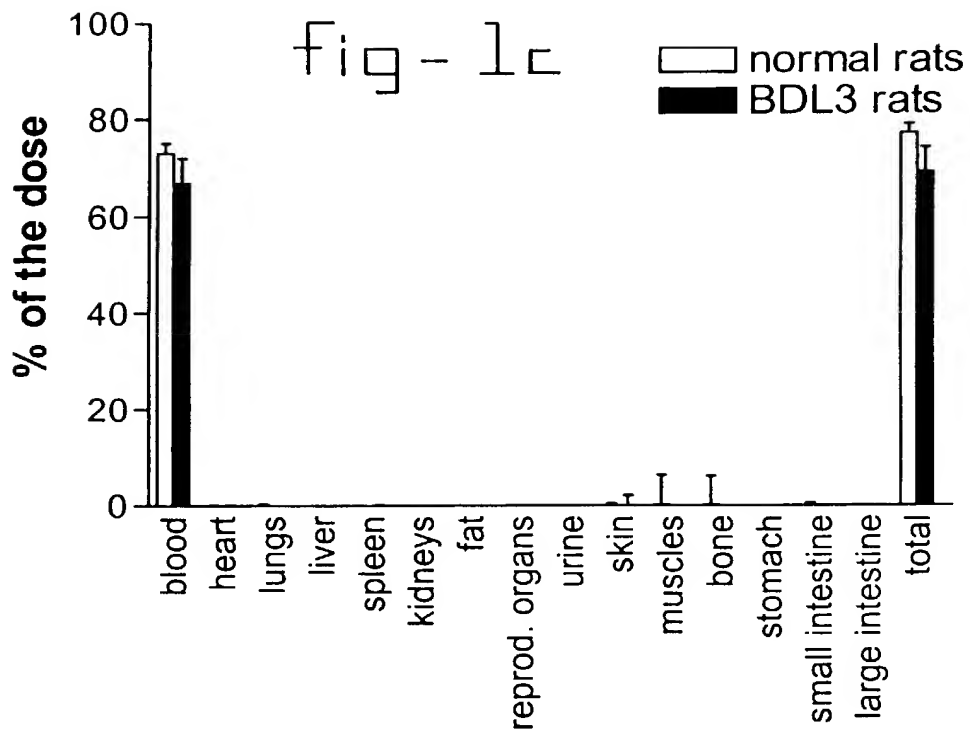
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/NL98/00579 (22) International Filing Date: 8 October 1998 (08.10.98) (71) Applicants (for all designated States except US): STICHTING VOOR DE TECHNISCHE WETENSCHAPPEN [NL/NL]; P.O. Box 3021, N-3502 GA Utrecht (NL). RIJKSUNIVERSITEIT TE GRONINGEN [NL/NL]; P.O. Box 72, NL-9700 AB Groningen (NL). (72) Inventors; and (75) Inventors/Applicants (for US only): POELSTRA, Klaas [NL/NL]; Kruidrad 2, NL-9285 MT Buitenpost (NL). BELJAARS, Eleonora [NL/NL]; Schoolstraat 27 A, NL-9712 JR Groningen (NL). MEIJER, Dirk, Klaas, Fokke [NL/NL]; Parklaan 17, NL-9724 AN Groningen (NL). SCHUPPAN, Detlef, Bruno, Igor [DE/DE]; Baumzeil 2, D-91088 Bubenreuth (DE). (74) Agent: DE BRUIJN, Leendert, C.; Nederlandsch Octrooibureau, Scheveningseweg 82, P.O. Box 29720, NL-2502 LS The Hague (NL).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: PEPTIDE-BASED CARRIER DEVICES FOR STELLATE CELLS (57) Abstract <p>The present invention relates to a compound comprising a carrier molecule, said carrier molecule being linked to a further molecule, said further molecule being at least one cyclic peptide, said cyclic peptide comprising in the cyclic peptide portion thereof at least one sequence encoding a cell receptor recognising peptide (RRP) and with the proviso that the compound is not a naturally occurring receptor agonist or antagonist. Preferably, the RRP is of a receptor specific for Hepatic Stellate Cells (HSC) or a receptor that is upregulated on HSC during disease. In particular, the RRP may be of a receptor selected from the group of PDGF receptor, collagen type VI receptor, cytokine receptor(s) such as TGFβ, IFNα and interleukin 1β. Preferably, the cyclic portion of the cyclic peptide comprises at least the amino acid sequence RGD or KPT. The compounds can be used as an active targeting ingredient for manufacturing a pharmaceutical composition for therapy, prophylaxis or diagnosis of a disease selected from the group consisting of fibrotic disease, sclerotic disease and chronic or acute inflammatory processes such as glomerulosclerosis, interstitial fibrosis, lung fibrosis, atherosclerosis, rheumatoid arthritis, Crohns disease, colitis ulcerosa, glomerulonephritis and sepsis, in particular for targeting HSC. The invention also relates to pharmaceutical compositions comprising the above compound(s).</p>		





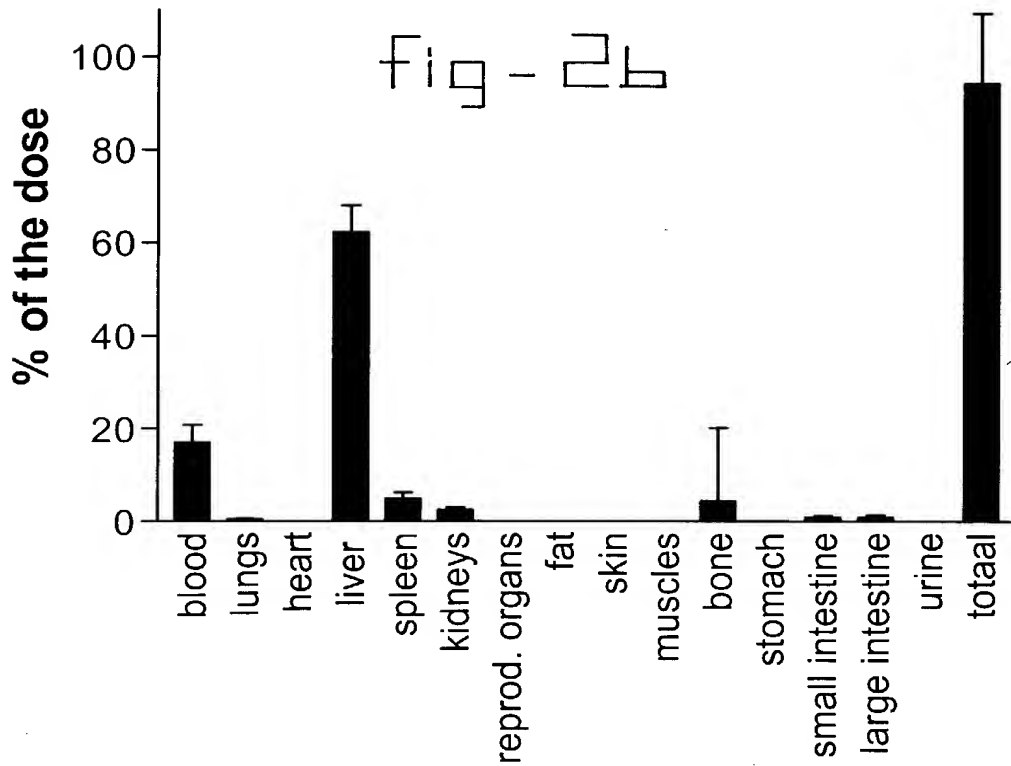
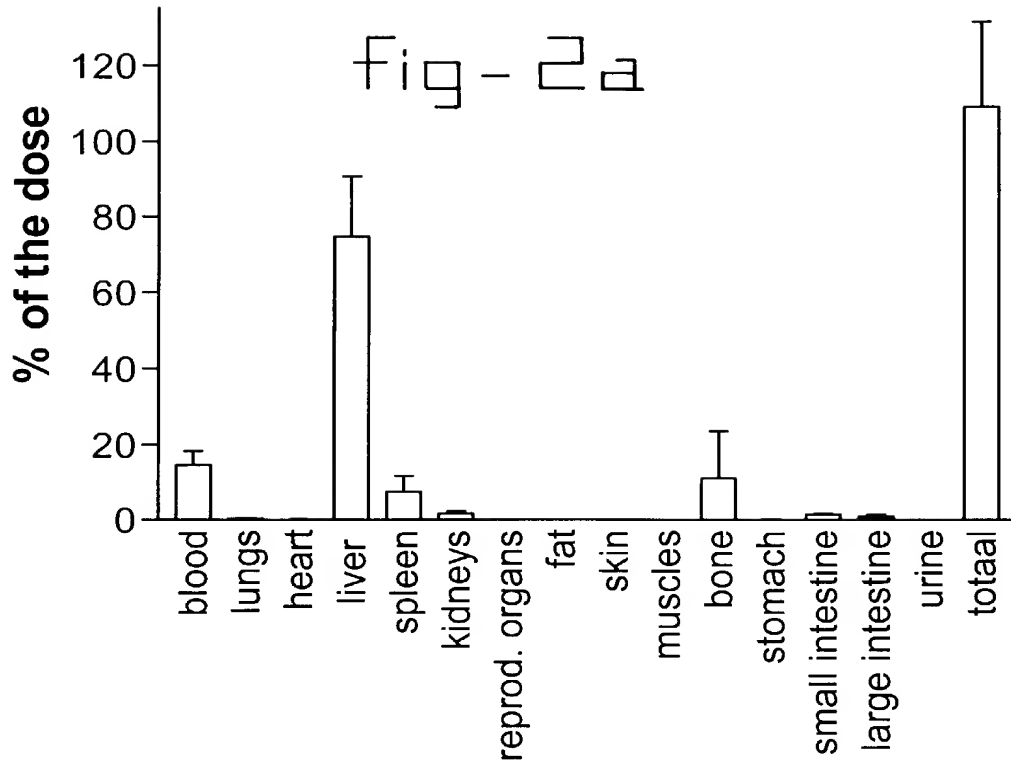


fig- 3a

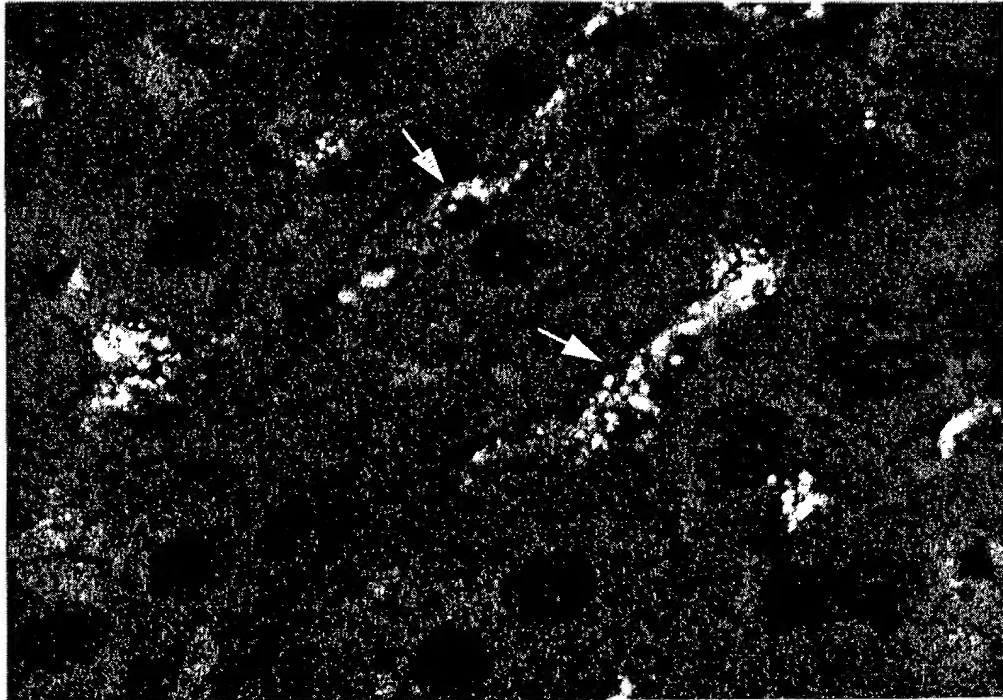
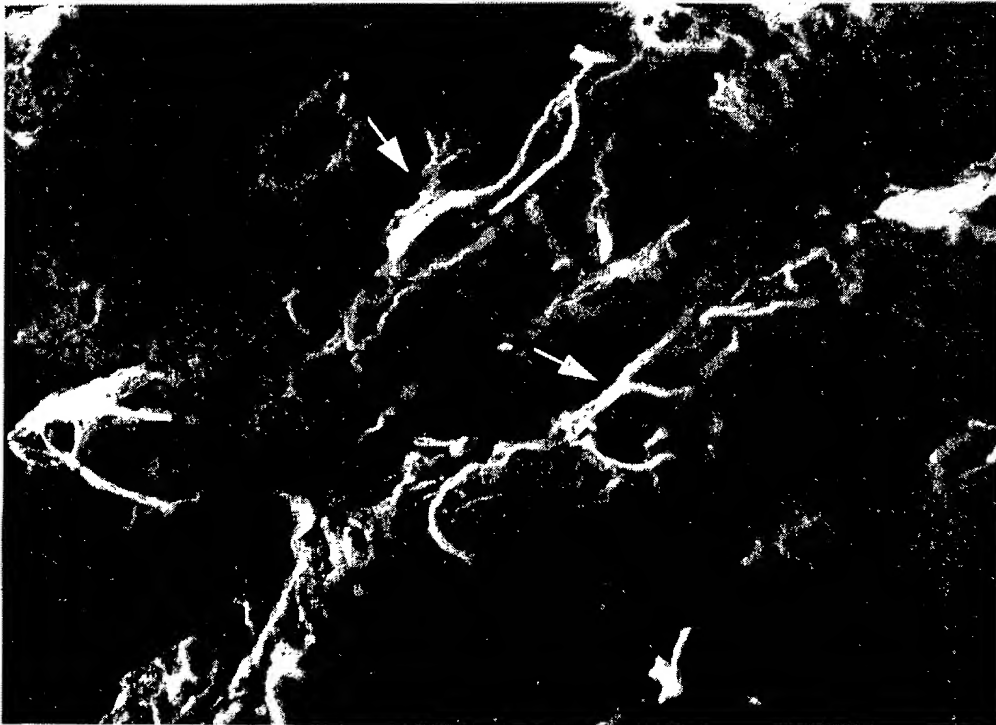


fig- 3b



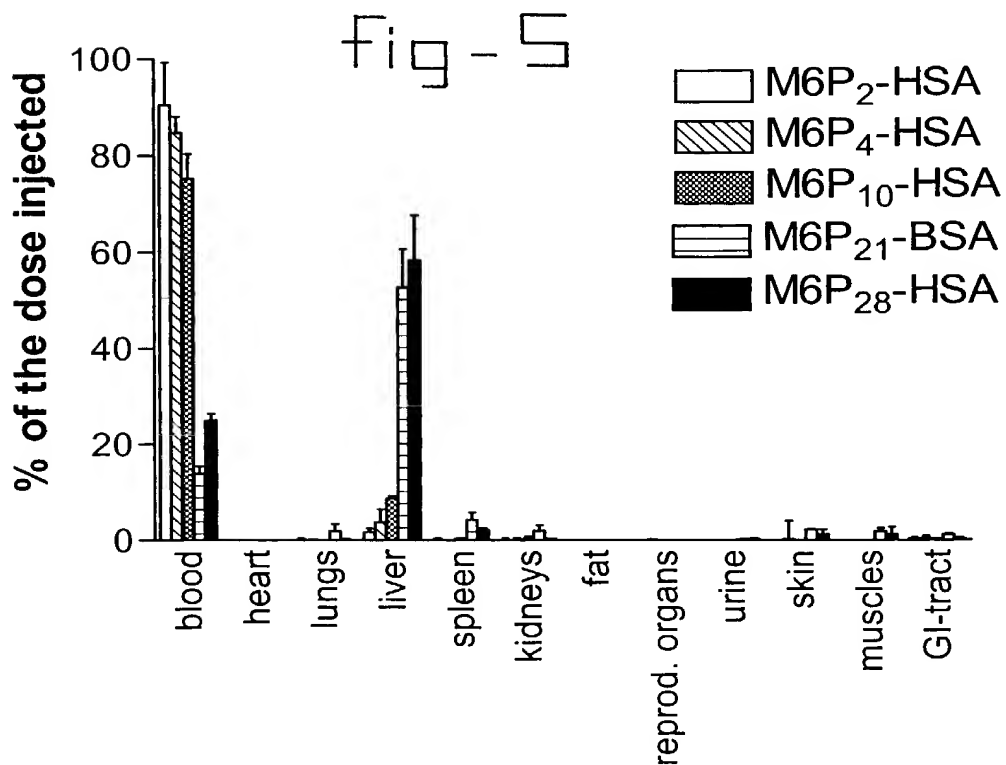
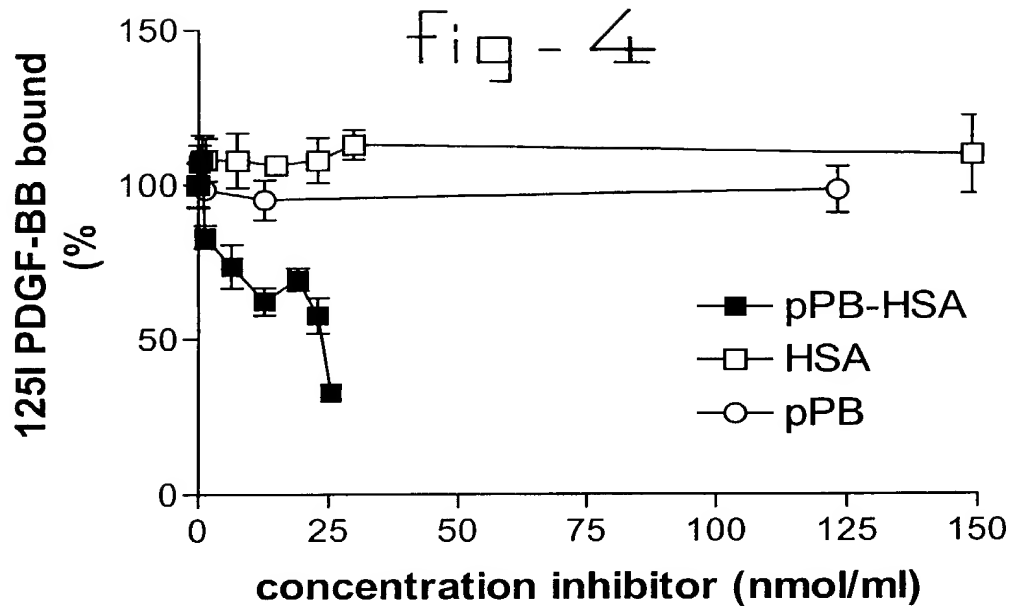
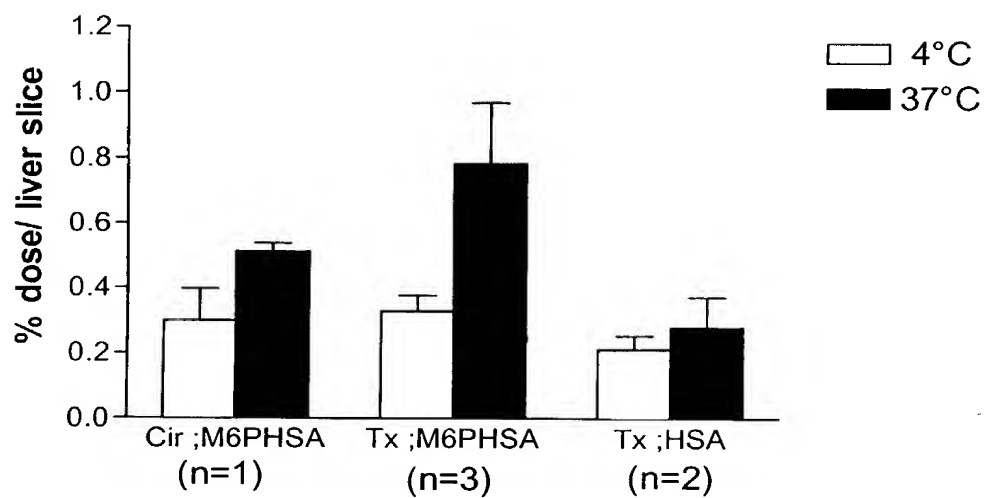


fig - 6





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21 MAY 2001

PCT #3

PATENT APPLICATION

Attorney Docket No. 7392/71228

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): POELSTRA et al.

Appln No.: 09/806,837

Filed: April 5, 2001

For: PEPTIDE-BASED CARRIER DEVICES FOR
STELLATE CELLS

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COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL DESIGN, NATIONAL STAGE OF PCT OR CIP APPLICATION)

As a below named inventor, I hereby declare that

My residence, post office address and citizenship are as stated below next to my name, I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Peptide-based carrier devices for stellate cells

the specification of which: (complete (a), (b) or (c) for type of application)

REGULAR OR DESIGN APPLICATION

- a. ☐ is attached hereto.
b. ☐ was filed on _____ as Application
Serial No. _____ and was amended on _____
(if applicable)

PCT FILED APPLICATION ENTERING NATIONAL STAGE

- c. ☒ was described and claimed in International application No. PCT/NL98/00579
filed on 8 October 1998
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ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, paragraph 1.56(a).

In compliance with this duty there is attached an information
disclosure statement 37 CFR 1.97

PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code paragraph 119 of any foreign application (s) for patent of inventor's certificate listed below and have also identified below any foreign application for patent of inventor's certificate having a filing date before that of the application on which priority is claimed.

(complete (d) or (e))

- d. ☐ no such applications have been filed
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(Complete this part only if this is a continuation-in-part application)

I hereby declare claim the benefit under Title 35, United States code, paragraph 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claim of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, paragraph 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, paragraph 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.) (Filing date) (Status) (patented, pending, abandoned)

(Application Serial No.) (Filing date) (Status) (patented, pending, abandoned)

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And I hereby appoint Cushman Darby & Cushman, L.L.P. 1100 New York Avenue, N.W., Ninth Floor, East Tower Washington, D.C. 20005-3918, telephone number 861-3000 (to whom all communications are to be directed), and the below-named persons (of the same address) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent, and I hereby authorize them to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/organization who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct Cushman, Darby & Cushman in writing to the contrary.

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1-00 Full name of sole or first inventor: POELSTRA, Klaas

Inventor's signature



Date 21 mei 2001

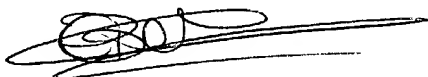
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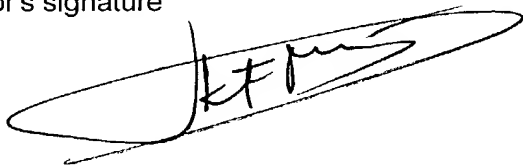
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